

## **REMARKS/ARGUMENTS**

Claims 59, 61-62, 72-74, and 77-78 are canceled without prejudice to renewal in a subsequently filed patent application. As a result of this Amendment and Remarks document, claims 51-58, 60, 63-71 and 75-76 are pending. In response to the examiner's remarks under the second paragraph of § 112, claims 51-53, 65-67 and 75 were amended to include the word "isolated". Additionally, "LTVEC" was moved in claims 51-53, 65-67, and 75 for improved readability. No new matter is added by these amendments, and the examiner is respectfully requested to enter them.

### **I. Formal matters.**

**A.** The specification was objected to for the presence of an embedded hyperlink at page 38, line 13. Accordingly, the specification is amended above to remove the hyperlink.

**B.** Claims 62, 74, and 78 were objected to for reciting both "non-human" and "mouse." This rejection is rendered moot by cancellation of the claims without prejudice to their renewal in a related patent application.

### **II. Double-Patenting Rejection**

**A.** Claims 51-78 were rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-14 of US 6,586,251 B2. Applicants agree to submit a terminal disclaimer upon indication of allowable claims.

**B.** Claims 51-78 were rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-14 of US 6,596,541 B2. Applicants agree to submit a terminal disclaimer upon indication of allowable claims.

### **III. Rejections under 35 USC § 112, first paragraph.**

Claims 51-78 were rejected on the basis that the specification enables mouse ES cells comprising a modified endogenous gene locus flanked by site-specific recombination sites, it does not enable non-mouse ES cells. This rejection is traversed as it applies to pending claims 51-58, 60, 63-71 and 75-76.

Applicants respectfully traverse this rejection on the grounds that one of skill in the art will readily recognize that the methods of the invention can be used to modify any eukaryotic cell. There is no requirement that the methods of the invention be restricted to ES cells or to mouse ES cells. A review of the scientific literature reveals that much research has been performed in which endogenous genes and chromosomal loci are variously modified in a wide assortment of eukaryotic cells. In fact, in the second paragraph of the first page in Chapter 1 in Joyner (1999) The Practical Approach Series 293:1-35, a well-known reference manual used by skilled artisans in the field of gene targeting (submitted in IDS dated 17 June 2002), the author states:

"The first experimental evidence for the occurrence of gene targeting in mammalian cells was made using a *fibroblast cell line* (emphasis added) with a selectable artificial locus by Lin, et al. and was subsequently demonstrated to occur at the endogenous  $\beta$ -globin gene by Smithies, et al. in *erythroleukemia cells* (emphasis added)."

For the examiner's convenience, the following publications are provided: Lin et al. (1985) Proc. Natl. Acad. Sci. USA 82:1391-1395, Smithies et al. (1985) Nature 317:230-234, Dai et al. (2002) Nature Biotechnology 20:251-255; Stivala et al. (2001) Oncogene 20:563-570; and Willers et al. (2000) Int. J. Radiat. Biol. 76:1055-1062, each of which describe research in which gene targeting was accomplished in non-ES cells.

The examiner has also stated that, "the state of the art is such that ES cell technology is generally limited to the mouse system at present." It is respectfully submitted that this statement is incorrect as it is based largely on relatively old scientific references (Moreadith, 1997; Mullins 1996) that do not accurately reflect the state of the art at the time this invention was made. Applicants herein provide the following scientific references that report the development of pluripotent ES cell lines from a variety of species including medakafish (Hong et al. (1998) PNAS USA 95(7):3679-84), rat (Lannaccone et al. (1994) Dev. Biol. 163(1):288-92), porcine (Chen et al. (1999) Theriogenology 52(2):195-212), rabbit (Schoonjans et al. (1996) Mol Reprod Dev 45(4):439-43), cynomolgous monkey (Suemori et al. (2001) Dev. Dyn. 222(2):273-9), rhesus monkey and marmoset (Thomson et al. (1998) Curr. Top Dev. Biol. 38:133-65), swine (Wheeler (1994) Reprod. Fertil. Dev. 6(5):563-8), and hamster (Doetschman et al. (1988) Dev. Biol. 127(1):224-7).

It is further respectfully pointed out that the methods of the invention require only that the vectors described herein be capable of homologous recombination within the target cell. It is well-established in the art that any cell that contains DNA is capable of homologous recombination as all DNA undergoes classic Watson and Crick-type base pairing. Furthermore, as was held in *In re* Bowen 492 F.2d 859, 181 USPQ 48 (C.C.P.A. 1974), even though the specification does not describe the use of all eukaryotic cells, including ES cells from every species, the examiner has not provided a persuasive reason why the specification does not realistically enable one skilled in the art to practice the invention as broadly as it is claimed, i.e. in any eukaryotic cell, including any ES cell. Even if the use of ES technology has not been perfected in some species, the methods of the present invention could certainly be used on any ES cell, or, for that matter, any eukaryotic cell as described above. Thus, applicants assert that there is no reason for the methods of the invention to be limited to mouse ES cells. Clearly, any eukaryotic cell will be amenable to the methods of the invention.

Accordingly, in light of the above remarks, it is respectfully requested that this rejection be withdrawn.

#### **IV. Rejections under 35 USC § 112, second paragraph.**

A. Claims 51-53, 65-67, and 75 were held to be unclear as the claims read on both *in vivo* and *in vitro* methods of replacing gene loci. It is suggested the claims be amended to recite "an isolated cell." In response, claims 51-53, 65-67, and 75 were amended as suggested by the examiner.

B. Claims 61, 72, and 77 were held to be unclear because they "do not recite that the ES cells are isolated." This rejection is rendered moot by cancellation of the claims.

C. Claims 59 and 73 were held to be unclear because they can refer to a gene or a gene inside of an organism. This rejection is rendered moot by cancellation of the claims.

D. Claims 62 and 78 were held to be incomplete for not reciting the steps between the ES cell and transgenic mouse. This rejection is rendered moot by cancellation of the claims without prejudice to their renewal in a related patent application.

#### **V. Rejections under 35 USC § 102(b).**

Claims 51-55, 57-63, 65-69, and 71-78 were rejected as anticipated by Kuncherlapati et al. (WO 94/02602). Claims 59, 61-62, 72-74 and 77-78 are canceled without prejudice or disclaimer. This rejection is respectfully traversed as it may be applied to claims 51-55, 57-58, 60, 63, 65-69, 71, and 75-76.

The legal test for anticipation requires that each and every element of the claimed invention be present in a single prior art reference. There is no anticipation under § 102 if a claimed element is excluded from a prior art reference. Atlas Powder Co. v. E.I. duPont de Nemours & Co., 224 U.S.P.Q.2d at 411 (Fed. Cir. 1984). Moreover, for a limitation to be considered "inherent," the single reference "must describe and enable the invention, including all the claim limitations, with sufficient clarity and detail to establish that the subject matter already existed in the prior art, and that its existence was recognized by persons of ordinary skill in the field of the invention ... *an inherent limitation is one that is necessarily present; invalidation based on inherency is not established by 'probabilities of possibilities.'*" Elan Pharmaceuticals Inc. v Athena Neurosciences, Inc., 304 F.3d 1221, 1228 (Fed. Cir. 2002); emphasis added).

The invention as claimed. The invention encompasses methods of creating a modified endogenous gene locus flanked either downstream, upstream, or both sides by a site specific recombination site, by creating the appropriate LTVEC(s), introducing the LTVEC(s) into an isolated eukaryotic cell, and using a quantitative assay to detect a modified cell. Introduction of the site specific recombination sites allows deletion of endogenous loci by introduction of a recombinase, which may further be replaced with a replacing gene. Deletions created using introduced site-specific recombination sites and recombinases may be much larger (up to at least 10 million base pairs) than those possible using single homologous targeting vectors. In one embodiment, the

method is used to modify an endogeneous immunoglobulin variable region gene locus, which may further be replaced with a human immunoglobulin variable region. Large targeting vectors (LTVECs) improve targeting efficiencies at sites difficult to target with other vectors, such as the immunoglobulin loci.

The cited prior art reference. Kuncherlapati et al, describe the introduction of standard targeting vectors (having homology arms less than 20 kb total) by homologous recombination or random integration of YACs, into ES cells.

Kuncherlapati et al. does not disclose or suggest (1) homologous recombination of large DNA vectors equivalent to a LTVEC (e.g., having homology arms that total greater than 20 kb), (2) targeted integration, (3) modifying an endogenous gene locus with site specific recombination sites, or (4) use of a quantitative assay to detect a modified cell.

The analysis under § 102(b). Applicants submit that the examiner has failed to establish a *prima facie* case of anticipation because the cited prior art reference fails to teach every limitation required by the claims. Although Kuncherlapati et al, describe the use of YACs, such YACs are introduced by random integration (see page 12, lines 25-29). Further, Kuncherlapati et al. fail to teach the use of quantitative assays, including quantitative PCR, to detect whether or not homologous recombination has occurred. The examiner states that Kuncherlapati et al. teach DNA analysis by Southern blot hybridization or (junctional) PCR. However, neither of assays are the quantitative assays required by the claims. Both junctional PCR and Southern blot assays detect correct targeting "qualitatively" by probing across the homology arms of the targeting vector. These methods are prohibitively difficult or impossible using LTVECs, because of the large lengths of homology. The quantitative MOA assay of the instant claims does not require probing across the homology arms of the vector. Instead, the MOA assay quantitatively determines that one of the native alleles has been modified or lost. In one example of the method of the invention, the quantitative MOA assay is used to distinguish between two native, non-modified alleles in non-targeted cells and a single native, non-modified allele in a targeted cell.

Still further, the instant claims require the creation of flanking site specific recombination sites, a feature not disclosed or suggested by Kuncherlapati et al. The insertion of site-specific recombination sites allows for the deletion or modification of the very large stretches of the genome, for instance, deletion of all of the mouse immunoglobulin heavy chain variable gene segments spanning approximately 1 million base pairs. Accordingly, in light of the above remarks, applicants respectfully request that this rejection be withdrawn.

## **VI. Rejections under 35 USC § 103(a).**

**A.** Claims 51-63 and 65-78 were rejected as obvious in light of Kuncherlapati et al. when taken with Yang et al. (1997) Nature Biotechnology 15:859-865. This rejection is respectfully



traversed. The claimed invention and Kuncherlapati et al. are summarized above.

Obviousness is a legal conclusion based on underlying facts of four general types: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the differences between the claimed invention and the prior art; and (4) any objective indicia of nonobviousness. See Graham v. John Deere Co., 383 U.S. 1, 17-18, 15 L. Ed. 2d 545, 86 S. Ct. 684 (1966); Continental Can Co. USA, Inc. v. Monsanto Co., 948 F.2d 1264, 1270, 20 USPQ2d 1746, 1750-51 (Fed. Cir. 1991); Panduit Corp. v. Dennison Mfg. Co., 810 F.2d 1561, 1566-68, 1 USPQ2d 1593, 1594 (Fed. Cir. 1987). Determination of obviousness cannot be based on the hindsight combination of components selectively culled from the prior art to fit the parameters of the patented invention." ATD Corp. v. Lydall, Inc., 159 F.3d 534, 546, 48 USPQ2d 1321, 1329 (Fed. Cir. 1998). There must be a teaching or suggestion within the prior art, within the nature of the problem to be solved, or within the general knowledge of a person of ordinary skill in the field of the invention, to look to particular sources, to select particular elements, and to combine them as combined by the inventor. See Ruiz v. A.B. Chance Co., 234 F.3d 654, 665, 57 USPQ2d 1161, 1167 (Fed. Cir. 2000); ATD Corp., 159 F.3d at 546, 48 USPQ2d at 1329; Heidelberger Druckmaschinen AG v. Hantscho Commercial Prods., Inc., 21 F.3d 1068, 1072, 30 USPQ2d 1377, 1379 (Fed. Cir. 1994) ("When the patented invention is made by combining known components to achieve a new system, the prior art must provide a suggestion or motivation to make such a combination.").

Yang et al. as a whole. Yang, et al, describe the random integration of BAC-derived vectors into ES cells. The vectors are not introduced into ES cells by homologous recombination; rather, the BACs are modified by bacterial homologous recombination to create BAC-derived vectors. These vectors are randomly integrated into the ES cells.

Yang et al. does not disclose or suggest (1) targeted integration, (2) the use of site specific recombination sites, or (3) use of a quantitative assay to detect a modified cell.

The analysis required by § 103(a). As shown by the above analysis, applicants respectfully submit that the examiner has failed to establish a *prima facie* case of obviousness because neither cited prior art reference, alone or in combination, suggest modifying an endogenous gene locus by methods which require (1) targeted integration, (2) the use of site specific recombination sites, or (3) use of a quantitative assay to detect a modified cell. Thus, even combining the cited references does not lead one to the claimed method.

Kuncherlapati et al. describe homologous recombination of standard targeting vectors into ES cells and random integration of large YACs into ES cells, and Yang et al. describe random integration of BAC-derived vectors into ES cells. Neither reference describes or suggests the introduction of BACs into ES cells by homologous recombination.

Further, neither reference describes the use of quantitative assays, including quantitative PCR, to detect whether or not homologous recombination has occurred. In fact, there was no need for Kuncherlapati et al. to use a quantitative assay to detect homologous recombination because, when using YACs, he obtained random integration (see page 12, lines 25-29), and when using targeting vectors, the homology arms were small enough for standard qualitative PCR or Southern blot analysis. Further, it was not the intention of Yang et al. to homologously recombine their BAC-derived vectors, so there was no need for them to assay for homologous recombination, either qualitatively or quantitatively. A review of both cited references reveals that the only analyses performed to assess vector integration into the host genome were exhaustive and time-consuming *qualitative* assays. See, for example, Kuncherlapati et al. at pages 43-45 describing the process of growing clones, isolating genomic DNA, digesting DNA, running agarose gels, performing Southern blots, probing blots, stripping blots, re-probing blots, analyzing the results. Many months are typically dedicated to performing such assays for *each* targeting experiment. In contrast, using the quantitative MOA assay of the instant invention allows identification of a modification made with any size fragment of DNA. By directly assaying cells using a MOA assay, one can determine whether or not the single allele of interest has been modified as desired within a period of a few hours. Use of a quantitative assay step is neither disclosed or suggested by either cited reference. Thus, there is no basis for stating that one of skill in the art could have had a reasonable expectation of success in arriving at the instant invention.

Still further, neither cited reference suggests the use of site specific recombination sites to delete and/or replace the deleted gene locus. Insertion of site specific recombination is highly advantageous for the precise engineering (deletion, replacement etc.) of megabase stretches of the genome, for instance, deletion of the approximately 2.8 million base pairs containing all of the mouse immunoglobulin kappa light chain variable gene segments while leaving the J chain and constant chain gene segments intact.

Accordingly, in light of the above remarks, it is respectfully requested that this rejection be withdrawn.

**B.** Claims 51-55, 57-69 and 71-78 were rejected as obvious in light of Kuncherlapati et al. when taken with Lie et al. (1998) Current Opinion Biotech 9:43-48. This rejection is respectfully traversed. The instant invention and Kuncherlapati et al. are summarized above.

Lie et al. as a whole. Lie et al. summarizes advances in quantitative PCR technology, for example, the Taqman® technology to quantify the number of copies of a DNA template in a genomic DNA sample.

Lie et al. do not disclose or suggest (1) methods of creating a modified endogenous gene locus flanked either downstream, upstream, or both sides by a site specific recombination site, (2)

homologous recombination of large DNA vectors equivalent to a LTVEC; or (3) targeted integration of an LTVEC into a host genome.

The analysis required by § 103(a). As described above, Kuncherlapati et al. do not disclose or suggest (1) homologous recombination of large DNA vectors equivalent to a LTVEC (e.g., having homology arms that total greater than 20 kb), (2) targeted integration, (3) modifying an endogenous gene locus with site specific recombination sites, or (4) use of a quantitative assay to detect a modified cell. Although the addition to Lie et al. addresses one of the missing elements, the combined references fail to teach the remaining missing elements.

Further, it is respectfully submitted that these references are not properly combinable because, as described above, Kuncherlapati et al. had no need to use a quantitative assay described by Lie et al. when using YACs, since he obtained random integration (see page 12, lines 25-29), and, when using targeting vectors, the homology arms were small enough for standard qualitative PCR or Southern blot analysis. Thus, there is no suggestion or teaching within the prior art references for making the combination.

Accordingly, in light of the above remarks, it is respectfully submitted that this rejection be withdrawn.

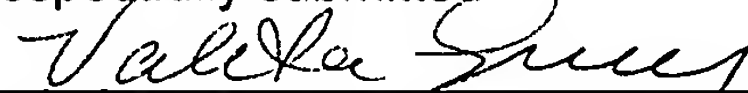
## **Conclusion**

It is believed that this document is fully responsive to the Office action dated 20 October 2004. In light of the above amendments and remarks, it is believed that the claims are now in condition for allowance, and such action is respectfully urged.

## **Fees**

Although it is believed that no fees are due, in the event the Patent Office determines that fees are due, the Commissioner is hereby authorized to charge Deposit Account Number 18-0650 in the amount of any fees deemed to be due.

Respectfully submitted



Valeta Gregg, Ph.D., Reg. No. 35,127  
Regeneron Pharmaceuticals, Inc.  
777 Old Saw Mill River Road  
Tarrytown, New York 10591  
(914) 345-7400